IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Examiner: Prebilic, P.

Tanagho, et al. DECLARATION UNDER RULE 131

Application No.: 08/994,83[illegible]

Filed: December 19, 1997

For: ACELLULAR MATRIX GRAFTS: PREPARATION AND USE

Assistant Commissioner for Patents
Washington, D.C. 20231
Sir:

We, E.A. Tanagho and R. Dahiya being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. Exhibit 1 attached hereto is incorporated herein by reference.

2. We completed the claimed invention detailed in the subject application in the United States prior to May 8, 1997. The work was done in our laboratories in California.

3. Enclosed is a copy of the journal cover page and article describing the work upon which the claimed invention is based. M. Probst *et al.*,(1997) Reproduction of functional smooth muscle tissue and partial bladder replacement, *British J. of Urology* **79**, 505-515. Figure two contains photographs of the matrix of the pending claims. The library circulation record is May 2, 1997 indicating that the public had possession of the journal by that date.

3. Declarants have nothing further to say.

Respectfully submitted,

E.A. Tanagho, M.D. E Tanagho Date 5/7/01

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EXHIBIT 1

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Reproduction of functional smooth muscle tissue and partial bladder replacement

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Objective To find a means of bladder augmentation that would avoid the complications encountered with the use of bowel segments, using a newly developed acellular biomaterial, the bladder acellular matrix graft (BAMG), as a homologous graft.

Materials and methods Thirty-four rats underwent a partial cystectomy (40–50%) and grafting with a BAMG of equal size. Eleven rats died within the first 72 h, probably from urinary leakage caused by obstruction of the bladder neck with stones or coagula; the surviving 23 were killed at varying intervals after cystectomy and examined.

Results After providing initial bladder enlargement, the graft was progressively infiltrated by the vessels and smooth muscle cells of the host; furthermore, the mucosal lining was complete within 10 days. After 4 weeks, all bladder wall components were evident histologically in the graft. The ingrowth was complete after 8 weeks, except for neural regeneration, which was only partial. At 12 weeks, the bladder wall muscle structure in the graft was so well developed that it was difficult to delineate the junction between host bladder and BAMG. Neural regeneration continued to improve. Normal bladder capacities were maintained throughout the study.

Conclusion The BAMG appears to serve, without rejection, as a framework of collagen and elastin for the ingrowth of all bladder wall components. The reason for the better acceptance of the BAMG than of other bladder augmentation grafts requires further investigation.

Keywords Urinary incontinence, homograft, acellular matrix graft, rat model

Introduction

The pathological changes of the detrusor muscle that result in contractile derangement may be congenital (epispadias, urethral valves or bladder exstrophy), inflammatory (interstitial, chronic or chemical cystitis, tuberculosis or schistosomiasis), traumatic (iatrogenic bladder destruction after surgery), functional (unstable or dysfunctional bladder) or radiation-induced (cancer of the cervix, rectum, prostate or bladder) [1,2].

The loss of contractility, poor compliance and the lack of muscular co-ordination can interfere with the bladder's reservoir function for the storage and periodic release of urine. This can affect the integrity of the entire urinary system and lead to recurrent urinary tract infections, incontinence, urolithiasis, renal parenchymal damage, renal impairment and failure [3].

After conservative forms of management have been exhausted, the therapeutic surgical treatment consists of three alternatives; urinary diversion with intestinal segments, augmentation or substitution cystoplasty. Disillusionment with the long-term results of cutaneous diversion [4,5] led to a preference for undiversionary procedures [6]. Almost all segments of large and small bowel have been tried for either augmentation or substitution cystoplasty, but the ideal segment that provides a continent low-pressure system and protects the upper urinary tract without side-effects has not been found [3,6].

In 1917, Neuhof [7] transplanted autologous fascia as an alternative augmentation material for enterocystoplasty in the urinary bladder in an animal model and in the short-term was able to show an increase in bladder capacity. Since then, many investigators have sought to achieve successful urinary bladder augmentation with a variety of auto-, homo- and heterologous tissues. These have included skin [8,9], preserved bladder [10], omentum [11], peritoneum [12], lyophilized human dura [13], chemically treated pericardium [14,15], and glutaraldehyde-treated amniotic membrane [16]. There were numerous reasons for failure, e.g. rejection of the graft, urinary tract infection, calculus formation, urinary fistulae, shrinkage and metabolic bone formation [2,11,14,16]. None of these materials ever showed the growth of the host bladder wall components into the graft.

The ideal augmentation material must serve initially as a sealant that will increase bladder capacity without rejection by the host. Over the long-term it must provide

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space for the progressive ingrowth of all host bladder wall components and finally become an integrated part of the bladder wall, with the same mechanical and functional properties as the host. In addition, it should be readily available at minimal expense without resulting in a second wound [2,15].

In recent years, two research groups have presented results that appeared to satisfy many of these prerequisites. In 1987, Fishman *et al.* [2] reported the use of human placental membrane for urinary bladder augmentation in the dog, with successful regeneration of normal smooth muscle in the area of the graft. Badylak *et al.* [17] have worked since 1989 with porcine small intestine submucosa (SIS), the end-product after abrading the tunica serosa, muscularis and mucosa. This consists mostly of collagen, elastin, muscularis mucosa and blood vessels and therefore has a paucity of cellular matter. In 1994, they reported the successful augmentation cystoplasty with SIS in an animal model [18]. The regenerated tissue showed epithelium, a basement membrane and smooth muscle cells.

This background excited our interest in enlarging the bladder by using a graft that consists mainly of acellular collagen and elastin, which serves as a scaffold for the ingrowth of smooth muscle tissue and mucosa. Instead of using porcine SIS, we took a full-thickness bladder dome in the rat, treated as described below, for a homologous transplant. The resultant bladder acellular matrix graft (BAMG) closely matches the mechanical, structural and genetic properties and size of the host bladder; in addition, there is no mechanical destruction during its production. The BAMG appears to be so markedly reduced in antigenicity that it could serve as either a homo- or a heterograft.

Materials and methods

Preparation of the BAMG

The bladder from a Sprague-Dawley rat (obtained from our institution's tissue-sharing programme) was excised and placed in a 35 mm Petri dish containing 50 mL of 10 mmol/L PBS (pH 7.0) and 0.1% sodium azide. The bladder was inverted and the mucosa scraped off with a pair of glass slides. The remaining lamina propria and detrusor muscle were treated with 50 mL of 10 mmol/L PBS-0.1% sodium azide and stirred for 5–6 h to allow partial cell lysis. The bladder was washed with 40 mL of PBS and then treated with 50 mL of 1 mol/L sodium chloride containing 2000 Kunitz units of DNase (Sigma, St Louis MO, USA) and stirred for 6–8 h. With this, lysis was complete and all the intracellular components were released. The samples were then treated with 50 mL of 4% sodium desoxycholate containing 0.1% sodium azide and stirred for 5–6 h to solubilize the lipid bilayer cell membrane and intracellular membrane lipids; this treatment was repeated once more. The resultant BAMG was washed three times with 50 mL PBS and stored in 10% neomycin sulphate at 4°C until grafted (Fig. 1).

Surgical technique

The rats were anaesthetized with intraperitoneal pentobarbital (40 mg/kg). Through a midline incision, the bladder was exposed, catheterized and filled with saline until fluid leaked around the urethral 3 F catheter (CR Bard Inc, Covington GA, USA). A hemicystectomy was performed without coagulation or ligation of the vessels (Fig. 2). The excised segment was saved for future preparation as an acellular matrix graft and the previously prepared BAMG was sutured in its place. The BAMG was trimmed to conform to the remaining host bladder and sutured in place with continuous monofilament 8/0 absorbable polyglycolic suture and four sutures of non-absorbable 7/0 Dermalon (anterior, posterior, left and right) to identify the matrix borders (Fig. 2). The grafted bladder was tested for leakage by catheterizing and filling it with saline until fluid leaked around the urethral catheter, and the capacity was measured. When the closure was satisfactory, the abdominal wall and the skin were closed and a drain was left for 24 h inside the abdominal cavity. No drugs were administered postoperatively.

Before the rats were killed the bladder was again catheterized and filled with saline to the point of leakage and the bladder capacity measured. The abdominal wall was opened through a midline incision and the bladder exposed. After both ureters were ligated, the bladder was filled with formalin through the urethra and fixed in a distended state; the catheter was removed and the urethra ligated. The specimen was excised *en bloc* (with the prostate and seminal vesicles in males and the ventral part of the uterus in females) and processed for histological examination.

Staining

The specimens for light microscopy were embedded in paraffin, sectioned and stained with trichrome for collagen and smooth muscle, haematoxylin and eosin (H&E) for nuclei, α-actin for smooth muscle and protein-gene product (PGP) for nerves.

Computer colour analysis

Bladder wall components may be evaluated qualitatively by describing the changes in the gross anatomical and histological cyto-architecture. In contrast, quantitative

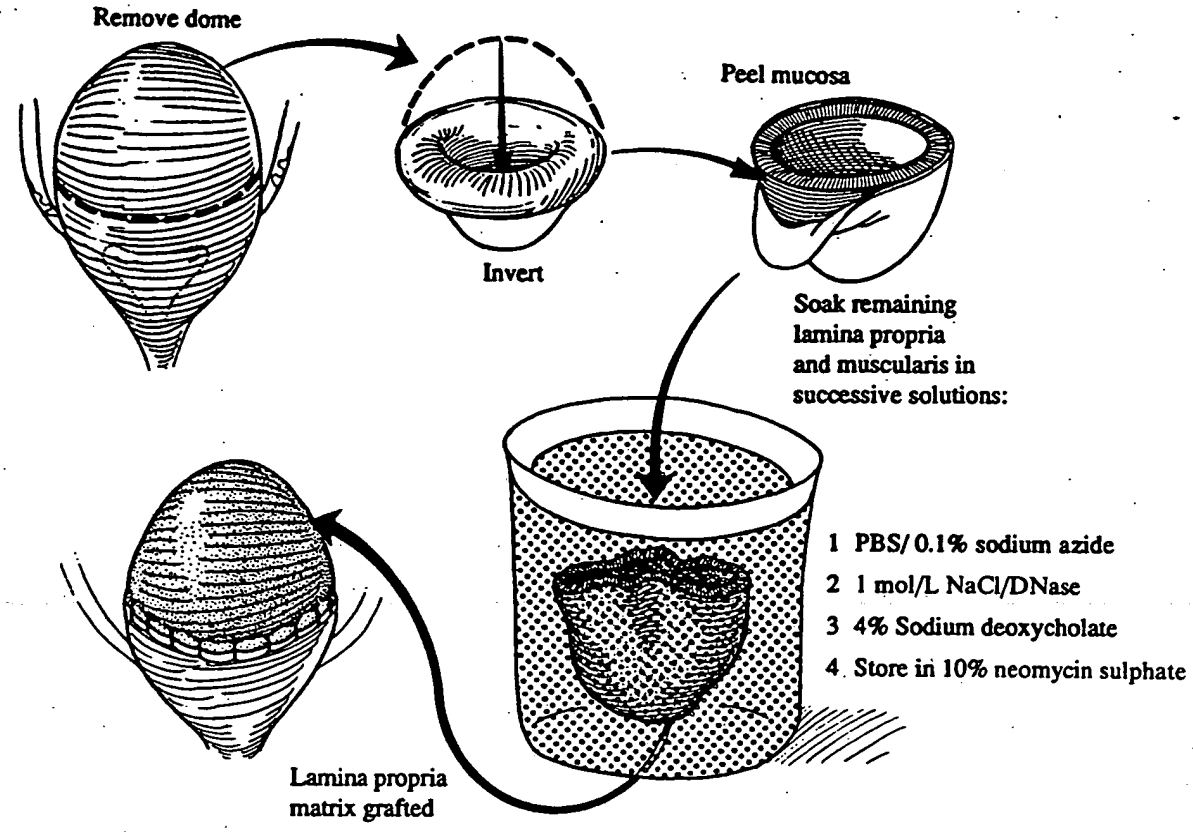

Fig. 1. Steps for processing a rat bladder dome into the BAMG (see Methods).

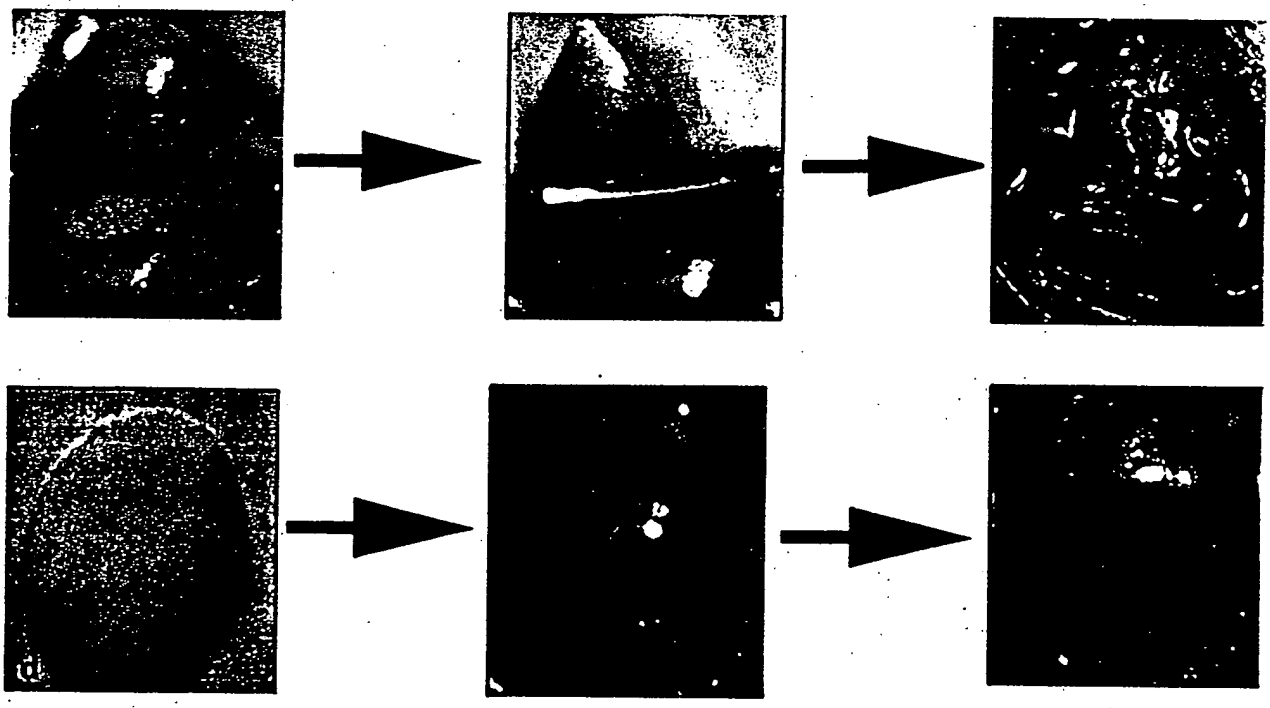

Fig. 2. a, Normal rat bladder; b, 50% cystectomy; c, rat bladder after hemicystectomy; d, the BAMG; e, rat bladder grafted with the BAMG; f, post-operative rat bladder (arrows indicate two identification sutures).

evaluation relies on destructive methods that necessitate the chemical destruction of the tissues in question [19]. In the present study, Adobe Photoshop™ was used for a computer-assisted morphological analysis to show both qualitative and quantitative changes.

For the ratio of smooth muscle to the remaining tissue, 10 slides from each bladder (stained for α-actin, × 100) were taken. Of these 10 slides, four represented the original bladder and six the matrix. For the analysis of the kind and amount of cellular infiltration during the first 14 days from cystectomy, six slides from each bladder (trichrome-stained, × 100) were taken; two slides represented the middle, two the right and two the left border of the matrix. The slides were digitized using a slide scanner (Polaroid Sprint Scan 35; resolution 600 d.p.i.) and the images processed with Adobe Photoshop™. The bladder wall component of interest (e.g. smooth muscle) was identified and other structures erased from the image (e.g. mucosa, erythrocytes inside the vessels, accessory tissue and background colouring left by the staining process). The number of pixels in the remainder was counted and set at 100%. The pixels in the colour range corresponding to the stain of the tissue type in question (e.g. with α-actin, brown was smooth muscle) were counted and expressed as a percentage of the total number of pixels. For the analysis of cellular infiltration, the number of pixels for leucocytes and lymphocytes was determined and expressed as a percentage of the total number of pixels.

Results

Of the 34 rats (19 male and 15 female) used for the study, 11 (eight male and three female) died 2–3 days after surgery from severe peritonitis consequent upon urinary extravasation caused by occlusion of the bladder neck, either by infectious bladder stones (nine) or blood coagula (two). The surviving 23 rats (11 male and 12 female) were killed at the following times; three each at 1 and 2 weeks, two at 4 weeks, one at 6 weeks, four each at 8 and 12 weeks, and three each at 16 and 20 weeks.

There were some adhesions on the peritoneal side of the graft between the matrix and especially the uterus and/or omentum. These occurred more frequently in the rats examined soon after surgery than in those examined later. Whether they represented a reaction to surgical trauma or a fusion between the matrix and the other tissues could not be determined.

After 2 weeks, small blood vessels were visible on the inside of the graft and after 4 weeks, on the outside. At 4 and 8 weeks, the reconstructed bladders appeared normal in size and shape. In nine of the surviving 23 rats, one to two bladder stones (magnesium, ammonium phosphate and calcium phosphate) were found. The mean (SD) bladder capacity was a little higher at death (2.5 [0.821] mL) than it was immediately after surgery (2.2 [0.514] mL).

Light microscopic examination of histological sections from the BAMG revealed an intact structure of collagen and elastin matrix with no evidence of nuclei (Fig. 3). Histologically, trichrome and H&E staining of the specimens of eight rats that died up to 3 days post-operatively showed an infiltration of the BAMG with erythrocytes and mononuclear cells (three rats were not included because death occurred more than 12 h before discovery). The red blood cells covered 10–20% and the mononuclear cells 2.5–6.5% of the grafted area (computer colour analysis). These cells were mainly at the border between the host bladder and graft as well as on the outside of the graft. The mucosa could not be evaluated because of post-mortem autolysis. Neither smooth muscle tissue nor nerve fibre growth was detectable.

In the surviving rats, trichrome and H&E staining of 1-week specimens also showed an infiltration of the BAMG with erythrocytes and mononuclear cells (Fig. 4). In the grafted area, 10% were red blood cells and $< 10\%$ mononuclear cells, mainly at the border between host and graft as well as at the luminal side of the graft. Computer colour analysis was not feasible because other kinds of cells had also invaded the area. The mucosa covered 40–50% of the luminal surface.

Alpha-actin staining at one week showed a pronounced capillary infiltration from the host bladder (Fig. 5). An infiltration of other α-actin-positive cells into the graft at its border with the host was also observed (Fig. 5), although it assumed no specific orientation; PGP staining was negative. After 2 weeks, the matrix graft was lined uniformly by several layers of urothelium and there was no difference from the urothelial lining of the host bladder (Fig. 6). Alpha-actin-positive cells (15%) were found inside the graft (Table 1) and organized spatial muscularization had begun (Fig. 6); there were many more vessels and PGP staining was still negative. After 4 weeks there was no change in the urothelial lining; the muscularization extended to 27% of the graft (Table 1; Fig. 7) and the number of capillaries had increased further. The first tiny nerves were apparent on PGP staining (Fig. 8). After 8 weeks, the amount of smooth muscle had increased to 35%, spatial orientation of the detrusor muscle had continued and, in some parts of the graft, differentiated muscularis mucosa was found (Fig. 9). Again, there were more capillaries and more and larger nerve fibres. After 12 weeks, the structure of the detrusor muscle and muscularis mucosa was so well developed and oriented (36% graft and 44% host) that it was sometimes difficult to delineate the junction

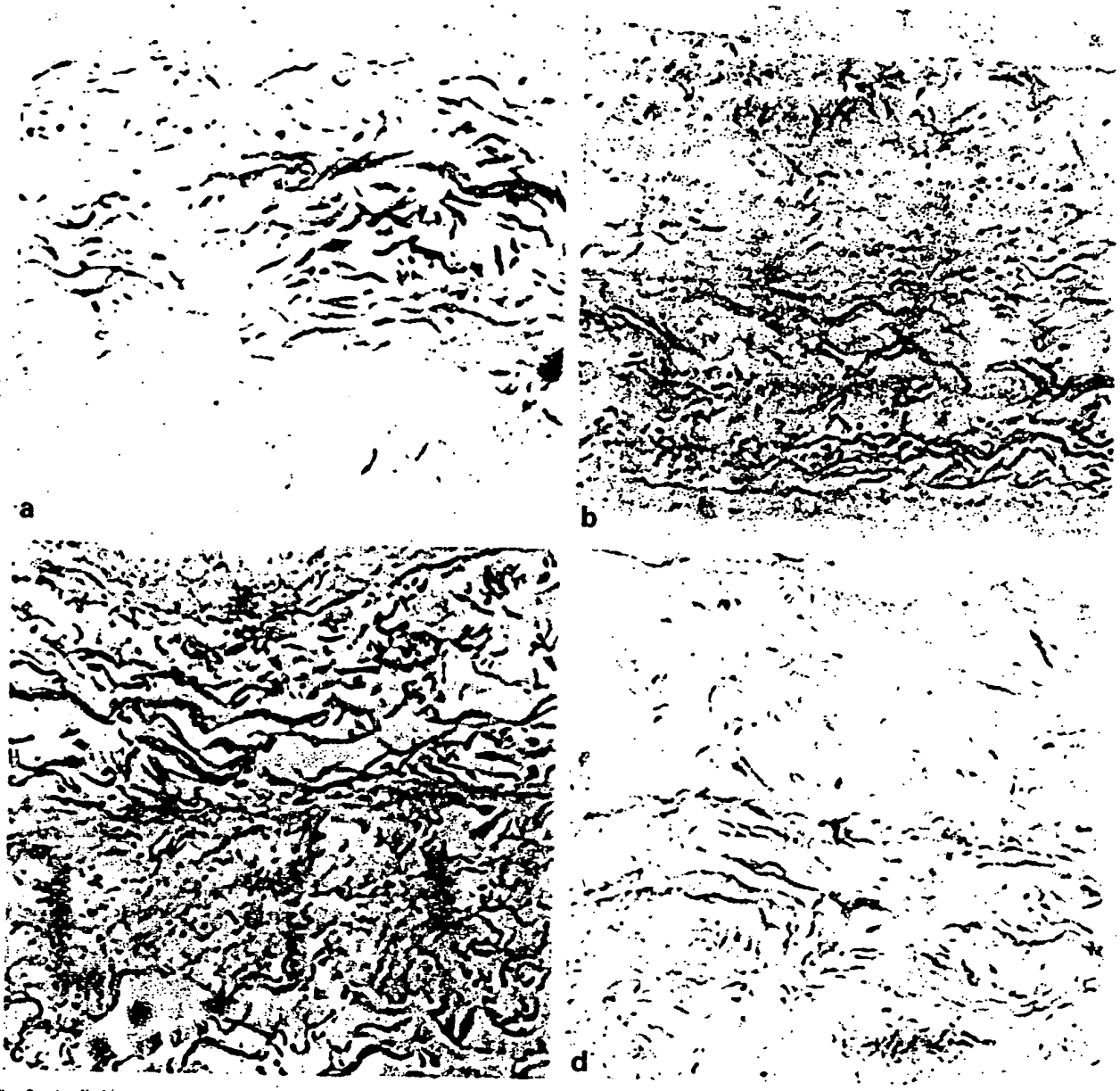

Fig. 3. Acellularity of the BAMG demonstrated by staining with, a, trichrome, b, haematoxylin and eosin, c, α-actin, and, d, protein-gene product. Stained spots in c are the result of cell fragments. ×400.

between the host bladder and BAMG. There were fewer capillaries, although the thickness of their walls had increased. The PGP staining showed yet more and larger nerve fibres (Fig. 10).

At 16 and 20 weeks, there were no major changes from the 12 week specimens. On histological examination, the urothelium, muscularis mucosa and detrusor muscle of the BAMG and host bladder appeared qualitatively similar (Fig. 11). The amount of α-actin-positive smooth muscle in the graft was only 17% less than in the host (Table 1). There were more vessels in the BAMG than in the host bladder; in contrast, there were fewer nerve fibres.

Discussion

The ultimate goal of bladder augmentation is the faithful regeneration, without adverse effects, of a muscular bladder wall lined on its luminal surface by epithelium. In the present study, this was achieved by transplantation of a BAMG to healthy rat bladder tissue. The transplant became integrated within the host bladder

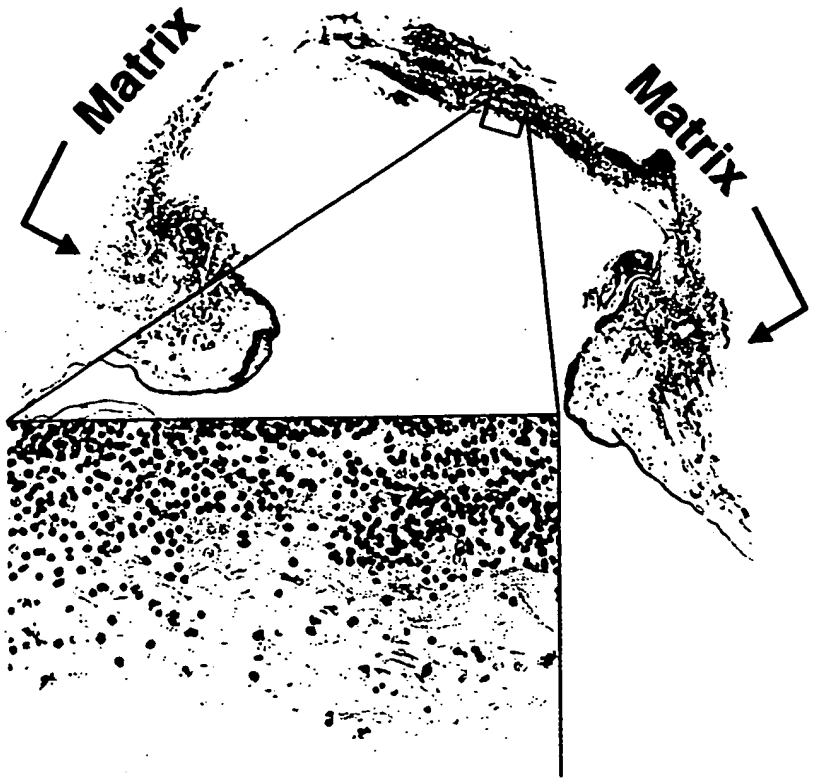

Fig. 4. The cellular infiltration in the 1 week graft consisted of 10% red blood cells and < 10% mononuclear cells. Trichrome. × 400. (Close-up shows that the cellular infiltration consists mostly of erythrocytes.)

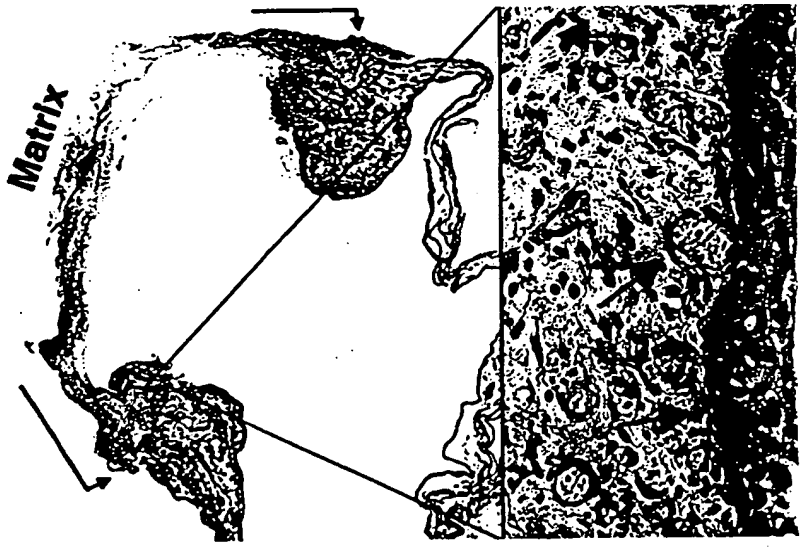

Fig. 5. Alpha-actin staining (× 400) in 1 week specimen shows pronounced capillary infiltration (black arrow) and non-oriented infiltration of other α-actin-positive cells (red arrow). The mucosa (green arrow) covers 30–40% of the luminal surface.

macro- and microscopically within 8 weeks. (The site of the matrix transplant could be clearly identified by the non-absorbable sutures.)

The acellularity of the graft was determined by staining sections with trichrome, H&E, α-actin and PGP; no evidence of nuclei was found.

The fairly high incidence of bladder calculi in this study (53%), which was primarily responsible (83%) for the early post-operative deaths, is based on several factors. First, the high incidence of stone formation (noted as early as 24 h) was an added risk factor in general [20,21]. Second, there are reports that bladder stones can be observed in 20–33% after augmentation cystoplasty with different autograft materials [22–24]. Third, the absence of the urothelial lining, and therefore of a mucus coating in the graft, could have increased the adherence of bacteria, calcium oxalate and urate crystals [25].

It is difficult to determine the grade of rejection in this acellular transplant, because all histological scoring

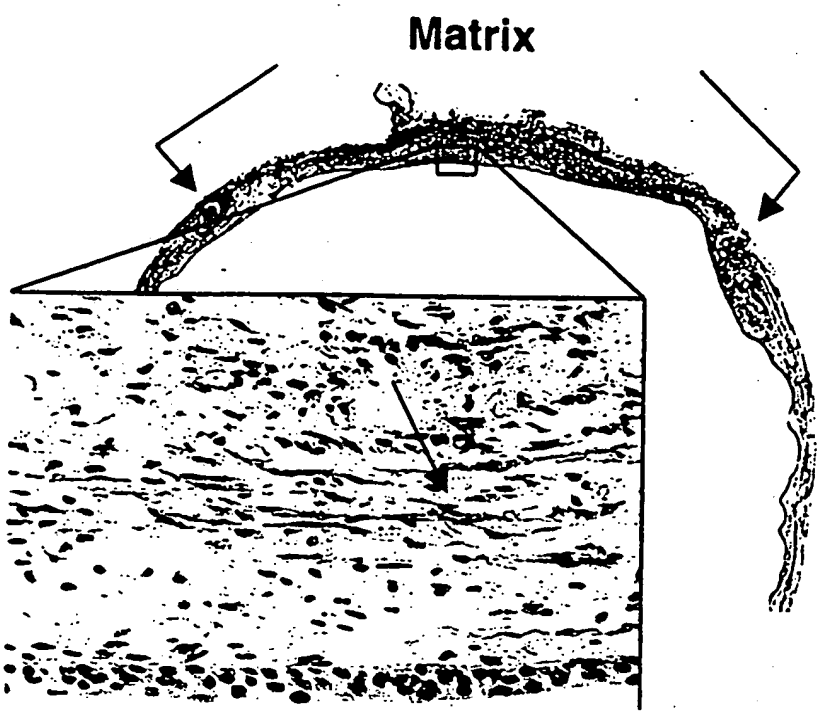

Fig. 6. After 14 days, the urothelium developed a uniform lining (yellow arrow) and the spatial orientation of the smooth muscle had begun (red arrow). Alpha actin. × 400.

Table 1 Progression of graft differentiation over time

	Time (weeks)						
Tissue	*1*	*2*	*4*	*8*	*12*	*16*	*20*
Mucosa	Mucosa complete (depending on the size of the graft)	Complete	Same as host	Same as host	Same as host	Same as host	Same as host
Detrusor muscle	Actin-stained cell infiltration	Smooth muscle cell orientation	Entire graft infiltrated by muscle cells	Spatial orientation in ↑ volume	Complete	Same as host	Same as host
Muscularis mucosa	–	–	–	Beginning of differentiation	Complete differentiation	Same as host	Same as host
Vessels	Beginning vascularization	↑↑ Vascularity	↑ Vascularity	Stable	↓ Number thicker walls	Stable	Graft more vascular than host
Nerve	–	–	Tiny nerves	↑ Number and size	Continued ↑	Continued ↑	Continued ↑

systems are based on cellular changes within the grafted material. Therefore, the only useful parameter is the infiltration of the graft with inflammatory cells. According to the literature [26], this infiltration consists for the first few days mostly of small lymphocytes, but the proportion of neutrophilic leucocytes increases with time such that by the ninth day these are found in about equal amounts. In the present study, the number of inflammatory cells inside the BAMG constituted <10% of the matrix area. These results were found in the three animals dissected after 1 week and in eight rats that died post-operatively. Based on this result, the immunoreaction of the host to the BAMG can be scored, according to the literature, as less than minor. All other cross-sections (4–20 weeks) showed a trivial number of leucocytes and lymphocytes and could be regarded as evidence of no rejection.

A progressive blood supply developed within 1 week. This neoangiogenesis is another indication of the good acceptance of the BAMG [26]. In parallel, there was an invasion of α-actin-stained cells; this mechanism of muscle regeneration is still unclear. Based on previous reports, it is most likely enhanced by the process of angiogenesis, as the numerous capillaries that penetrate the graft carry with them pericytes that accompany the capillary endothelial cells. These pericytes have been

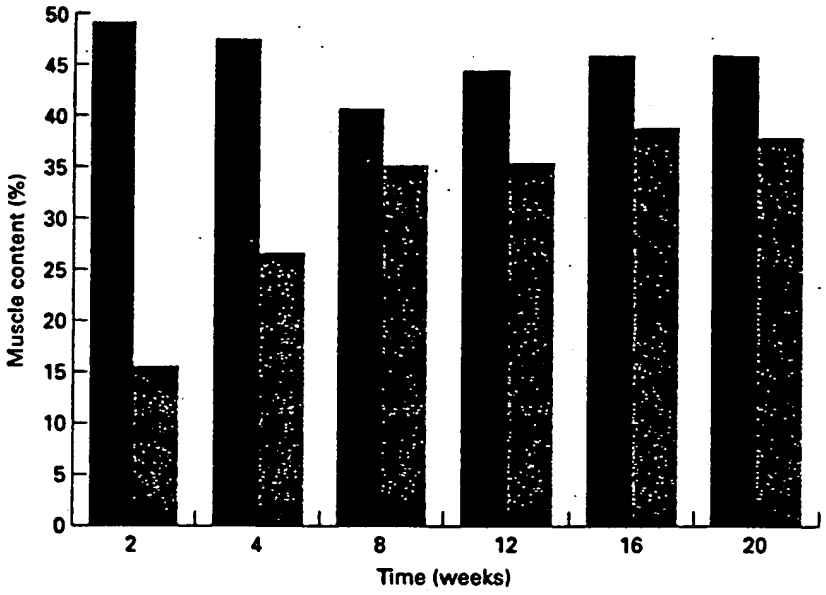

Fig. 7. The smooth muscle content in the BAMG versus the host bladder with time. Green, Host bladder. Red, Matrix bladder.

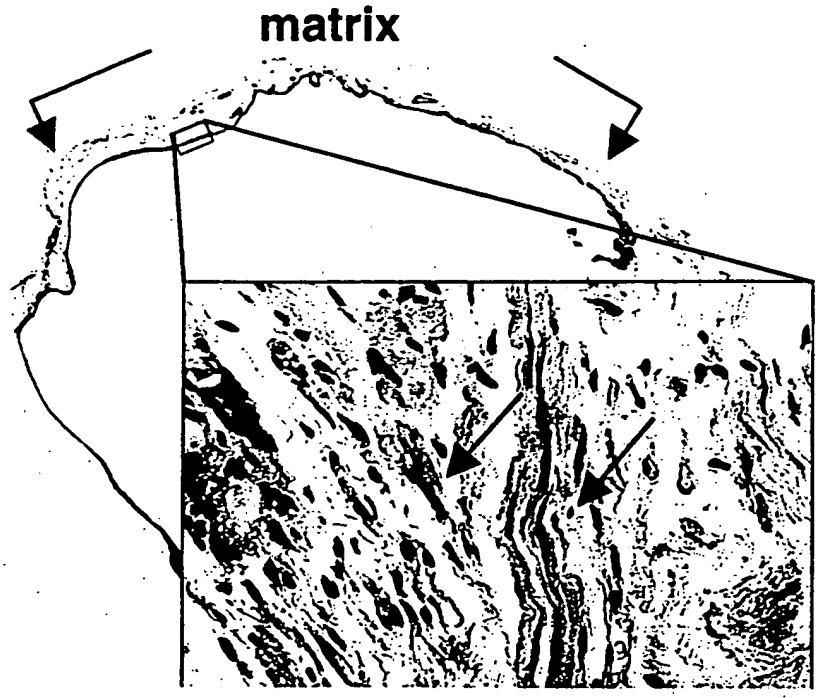

Fig. 8. In the 4 week graft, the first tiny nerves (black arrows) are apparent. PGP. × 400.

observed to contain intermediate filaments that are seen in smooth muscle cells [27]. That the literature has shown strong evidence that pericytes can be transformed into smooth muscle cells supports the hypothesis that these pericytes are the origin of muscularization of the BAMG.

Growth of the epithelial lining was clearly evident in all these studies [2,9,11–15] and could be the factor responsible for maintaining the integrity of the transplants. However, in the BAMG it might permit the ingrowth of fine capillaries and with them smooth muscle cells (pericytes) from the host bladder. The epithelialization provides maintenance for the BAMG. There were fewer nerve fibres in the BAMG, even after 20 weeks, than in the host. The reason for this slow regeneration of nerve fibres is unknown, although the distance that the nerve fibres must cross during their regeneration could be a factor [28].

The reason for the good acceptance of the BAMG compared with other bladder augmentation grafts is unclear. Previous reports have not shown significant ingrowth of bladder wall components. In all these

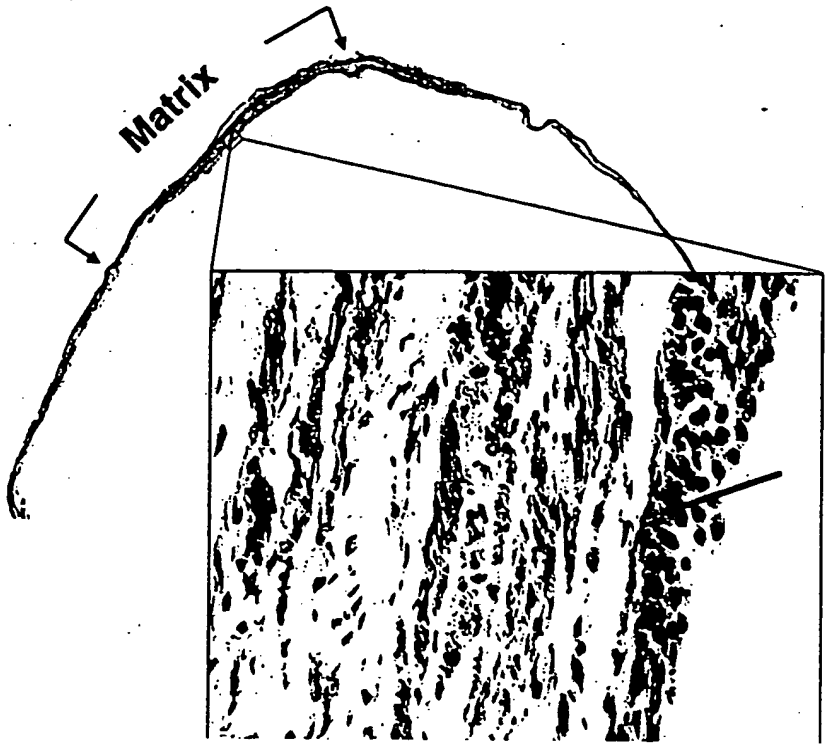

Fig. 9. In the 8 week graft, spatially oriented, well-developed detrusor muscle and a differentiated muscularis mucosa (black arrow) become obvious. Alpha actin. × 400.

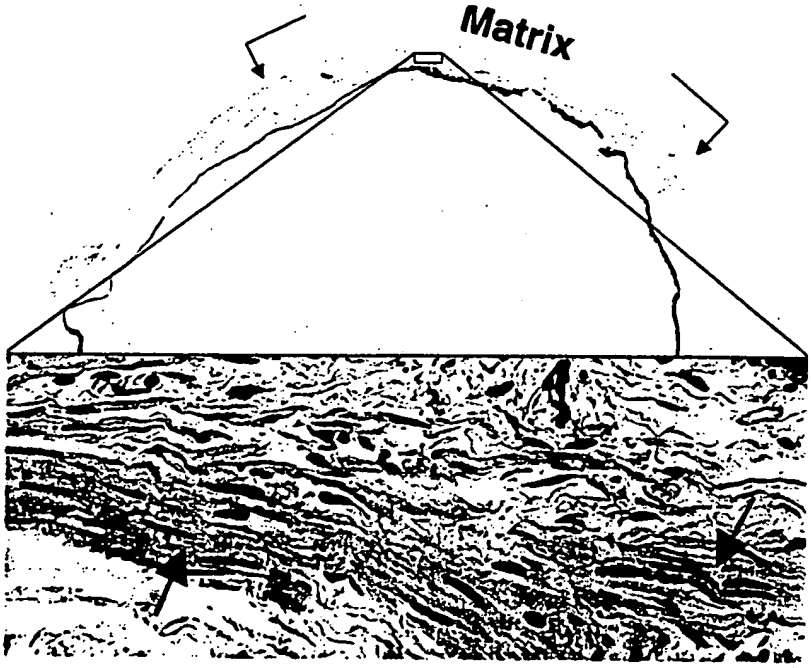

Fig. 10. By 12 weeks, there are more and larger nerve fibres (black arrow). PGP. × 400.

experiments, the intraluminal surface was covered with mucosa within 4–6 weeks (depending on the size of the graft and the animal model). The detrusor muscle and muscularis mucosa stopped at the edge of the transplant [7–16].

This preliminary study of the outcome of the homografted acellular rat bladder matrix shows evidence of dramatically decreased antigenicity, complete luminal epithelialization, rapid angiogenesis, perfect spatial reconstruction of detrusor muscle as well as muscularis mucosae, and regeneration of nerve fibres. There was no evidence for scar formation at the site of anastomosis. More work must be done to prove that these bladder components are truly functional and that they can work

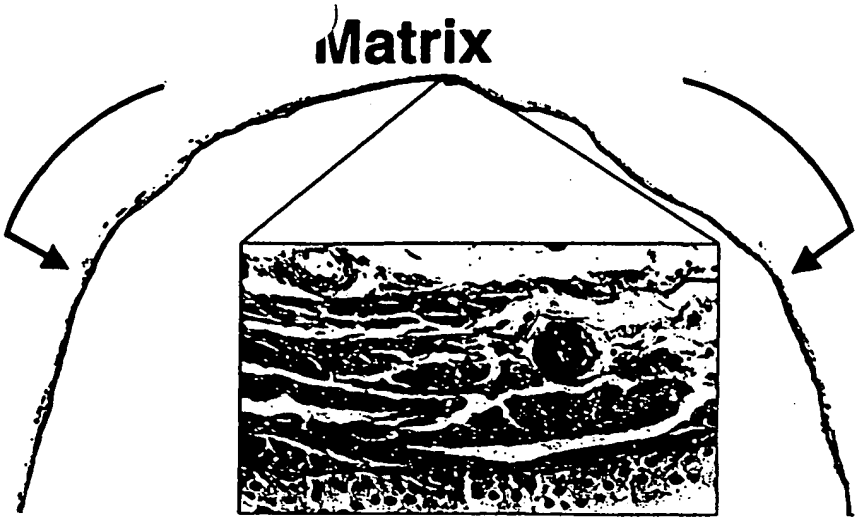

Fig. 11. The 20-week graft appears qualitatively similar in bladder wall structure to the host. Trichrome. × 400.

in co-ordination with the host components under the same neural influence and generate adequate intravesical pressure to produce sustained voiding with complete emptying. Detailed functional studies and experiments to assess the functional, biochemical and biophysical properties of the BAMG before and after surgery will be the subject of separate reports. We also hope to show that, because of the decreased antigenicity, homotransplants are feasible without immunosuppression.

Acknowledgements

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References

1 Goldwasser B, Webster GD. Augmentation and substitution enterocystoplasty. *J Urol* 1986; 135: 215–24
2 Fishman IJ, Flores FN, Scott FB, Spjut HJ, Morrow B. Use of fresh placental membranes for bladder reconstruction. *J Urol* 1987; 138: 1291–4
3 McDougal WS. Metabolic complications of urinary intestinal diversion. *J Urol* 1992; 147: 1199–208
4 Dunn M, Roberts JB, Smith PJ, Slade N. The long-term results of ileal conduit urinary diversion in children. *Br J Urol* 1979; 51: 458–61
5 Koch MO, McDougal WS, Hall MC, Hill DE, Braren HV, Donofrio N. Long-term metabolic effects of urinary diversion: a comparison of myelomeningocele patients managed by clean intermittent catheterization and urinary diversion. *J Urol* 1992; 147: 1343–7
6 Goldwasser B, Barrett DM, Webster GD, Kramer SA. Cystometric properties of ileum and right colon after bladder augmentation, substitution or replacement. *J Urol* 1987; 138: 1007–8
7 Neuhof H. Fascia transplantion into visceral defects. *Surg Gynecol Obstet* 1917; 14: 383–427
8 Draper JW, Stark RB. End result in the replacement of mucous membrane of the urinary bladder with thick-split grafts of skin. *Surgery* 1956; 39: 434–40
9 Pust R. [Urinary bladder plastic enlargement with the use of autologous, homologous and heterologous skin transplants]. *Fortschr Med* 1979; 97: 561–6
10 Tsuji I, Ishida H, Fujieda J. Experimental cystoplasty using preserved bladder graft. *J Urol* 1961; 85: 42–4
11 Goldstein MB, Dearden LC, Gualtieri V. Regeneration of subtotally cystectomized bladder patched with omentum: an experimental study in rabbits. *J Urol* 1967; 97: 664–8
12 Jelly O. Segmental cystectomy with peritoneoplasty. *Urol Int* 1970; 25: 236–44
13 Kelami A. Lyophilized human dura as a bladder wall substitute: experimental and clinical results. *J Urol* 1971; 105: 518–22
14 Novick AC, Straffon RA, Koshino I, Banowsky LH, Levin H, Kambic H. Experimental bladder substitution using a biodegradable graft of natural tissue. *J Biomed Mater Res* 1978; 12: 125–47
15 Kambic H, Kay R, Chen JF, Matsushita M, Harasaki H, Zilber S. Biodegradable pericardial implants for bladder augmentation: a 2.5-year study in dogs. *J Urol* 1992; 148: 539–43
16 Norris MA, Cohen M, Warren MM, Becker SN, Baur PJ, Seybold HM. Bladder reconstruction in rabbits with glutaraldehyde-stabilized amniotic membranes. *Urology* 1982; 19: 631–5
17 Badylak SF, Lantz GC, Coffey A, Geddes LA. Small intestinal submucosa as a large diameter vascular graft in the dog. *J Surg Res.* 1989; 47: 74–80
18 Knapp PM, Lingeman JE, Siegel YI, Badylak SF, Demeter RJ. Biocompatibility of small-intestinal submucosa in urinary tract as augmentation cystoplasty graft and injectable suspension. *J Endourol* 1994; 8: 125–30
19 Uvelius B, Lindner P, Mattiasson A. Collagen content in the rat urinary bladder following removal of an experimental infravesical outlet obstruction. *Urol Int* 1991; 47: 245–9
20 Deerberg F, Rehm S, Jostmeyer HH. Spontaneous urinary bladder tumors in DA/Han rats: a feasible model of human bladder cancer. *J Natl Cancer Inst* 1985; 75: 1113–21

21 Osanai T, Miyoshi I, Hiramune T, Ka[illegible]. Spontaneous urinary calculus in young LEW [illegible]ats caused by *Corynebacterium renale*. *J Urol* 1994; 152: 1002–4
22 Haselhuhn GD, Kropp KA, Keck RW, Selman SH. Photochemical ablation of intestinal mucosa for bladder augmentation. *J Urol* 1994; 152: 2267–71
23 de Badiola F, Manivel JC, Gonzalez R. Seromuscular enterocystoplasty in rats. *J Urol* 1991; 146: 559–62
24 Spencer JR, Steckel J, May M, Marion D, Hernandez K, Vaughan EJ. Histological and bacteriological findings in long-term ileocystoplasty and colocystoplasty in the rat. *J Urol* 1993; 150: 1321–5
25 Grenabo L, Hedelin H, Hugosson J, Pettersson S. Adherence of urease-induced crystals to rat bladder epithelium following acute infection with different uropathogenic microorganisms. *J Urol* 1988; 140: 428–30
26 Kuusanmaki P, Halttunen J, Paavonen T, Pakarinen M, Luukkonen P, Hayry P. Acute rejection of porcine small bowel allograft. An e[illegible]ed histological scoring system. *Transplantation* 1994; 5[illegible]. 757–63
27 Nehls V, Denzer K, Drenckhahn D. Pericyte involvement in capillary sprouting during angiogenesis in situ. *Cell Tissue Res* 1992; 270: 469–74
28 Lundborg G, Dahlin LB, Danielsen N *et al*. Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. *Exp Neurol* 1982; 76: 361–75

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